

## GENETIC VARIANTS OF CASEIN IN INDIAN AND AFRICAN ZEBU CATTLE

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**Abstract**—1. The caseins present in the milk of cows of five Indian and two East African Zebu breeds were examined by gel-electrophoretic procedures.

2. Like those of Western breeds, the  $\alpha_{s1}$ -,  $\beta$ - and  $\kappa$ -casein systems were polymorphic.

3. Most of the genetic variants appeared to be identical to those seen in Western breeds, but a variant ( $\beta$ -casein B<sub>2</sub>) of the same electrophoretic mobility as the  $\beta$ -casein B of Western cattle proved to be different in amino acid composition.

4. A rare new variant,  $\beta$ -casein D, was found in the milk of Indian Deshi and East African Boran cows.

5. Zebu breeds had a very high gene frequency of  $\alpha_{s1}$ -casein C, in contrast to Western breeds in which the B-variant predominates.

6. The East African milks were additionally examined for, but did not contain,  $\beta$ -lactoglobulin C, a variant characteristic for the Jersey breed.

### INTRODUCTION

THE PRINCIPAL components of the casein complex of cow's milk,  $\alpha_{s1}$ -,  $\beta$ - and  $\kappa$ -caseins, are each known to occur in the form of two, or more, variants inherited in a straightforward Mendelian manner (Aschaffenburg, 1965). Phenotyping studies have revealed breed differences in the occurrence and frequency of occurrence of the genetic variants, differences which, it is hoped, will be of help in clarifying the origin of, and relationship between, breeds. Virtually all the relevant data at present available concern the caseins of Western (*Bos taurus*) breeds of cattle. The caseins of a few milks of Indian Zebu (*Bos indicus*) cows have been examined by the rather insensitive technique of paper electrophoresis (Aschaffenburg & Sen, 1963). No evidence of polymorphism was found, but it was pointed out at the time that the number of samples tested was too small to preclude the existence of variants. That polymorphisms do, in fact, exist is shown by the more extensive data on the caseins of Indian Zebu breeds obtained with the aid of the searching gel-electrophoretic phenotyping procedures now available. In addition, phenotyping results are reported for two East African breeds of Zebu cattle.

## RESULTS

The electrophoretic patterns in alkaline gels of the caseins of Zebu cattle, illustrated in Fig. 1, were found to be very similar to those of Western breeds: all but one of the bands seen in Zebu patterns have their counterpart in those of Western breeds, and it is tempting to presume their identity. However, components of apparently identical electrophoretic mobility may differ in their amino acid composition or sequence. Thus one of us (M. P. T.) found three distinct differences in the peptide maps prepared from chymotryptic digests of the  $\beta$ -B caseins isolated from the milk of Indian Zebu and Western cows respectively. To indicate this divergence, the notation  $\beta$ -B<sub>Z</sub> will be used for the  $\beta$ -variant of Zebu cattle. There were no such differences in similar comparisons of  $\beta$ -casein A<sup>2</sup> and  $\alpha_{s1}$ -casein C, the variants predominating in Indian Zebu cows, suggesting that  $\beta$ -casein B<sub>Z</sub> is the exception rather than the rule. All other components will therefore be described in the terms accepted for the casein variants of Western cows (Thompson *et al.*, 1965).

One component in the Zebu pattern has no counterpart in that of Western breeds. This is the slowest-moving band of  $\beta$ -casein (Nos. 1 and 8, Fig. 1) which was at first thought to be  $\beta$ -casein C, but proved to be a new variant which we propose to call D. First seen in samples from Indian Deshi cows, it was later also encountered in samples from East African Boran cattle. The non-identity of D and C is clearly demonstrated in Fig. 2 which also illustrates the identical electrophoretic mobilities of some of the other casein variants of Zebu and Western cows. How the new variant differs in amino acid composition from that of the other known variants of  $\beta$ -casein is now being investigated.

The phenotyping results for the three casein components, as obtained by gel electrophoresis in alkaline buffer systems, are summarized in Table 1. Agreement between the observed numbers and those calculated from the Hardy-Weinberg law is so close that  $\chi^2$ -values have been omitted; the highest value, for the  $\kappa$ -casein of Deshi cows, does not exceed 2.5. There can be no doubt that, in Zebu as in Western cattle, the three casein components represent polymorphic systems. Qualitatively Zebus differ from Western cows in the production of  $\beta$ -casein B<sub>Z</sub>, and in the occurrence of the new variant  $\beta$ -casein D, quantitatively the most striking difference is the predominance of the C-variant of  $\alpha_{s1}$ -casein, as will be discussed later.

On electrophoresis in acid gel media, applied to only part of the material listed in Table 1, two variants of  $\beta$ -casein A were observed which corresponded in mobility to the variants A<sup>1</sup> and A<sup>2</sup> of Western breeds; the third, corresponding to A<sup>3</sup> was not seen. This variant has so far been found only in the milk of Holstein-Friesian cows (Kiddy *et al.*, 1966). As shown in Fig. 3,  $\beta$ -casein D cannot be differentiated from  $\beta$ -casein B<sub>Z</sub> or, for that matter, B under acid conditions, but this is no serious drawback, as electrophoresis under these conditions can only be regarded as a supplement to, but not a substitute for, electrophoresis in alkaline media, failing, as it does, to provide resolution of the  $\alpha_{s1}$ - and  $\kappa$ -caseins.

Examination of the 130 samples of total caseins from Indian cows by acid PAE showed that 126 carried the A-gene, no less than 124 in the form of the A<sup>2</sup>-variant.

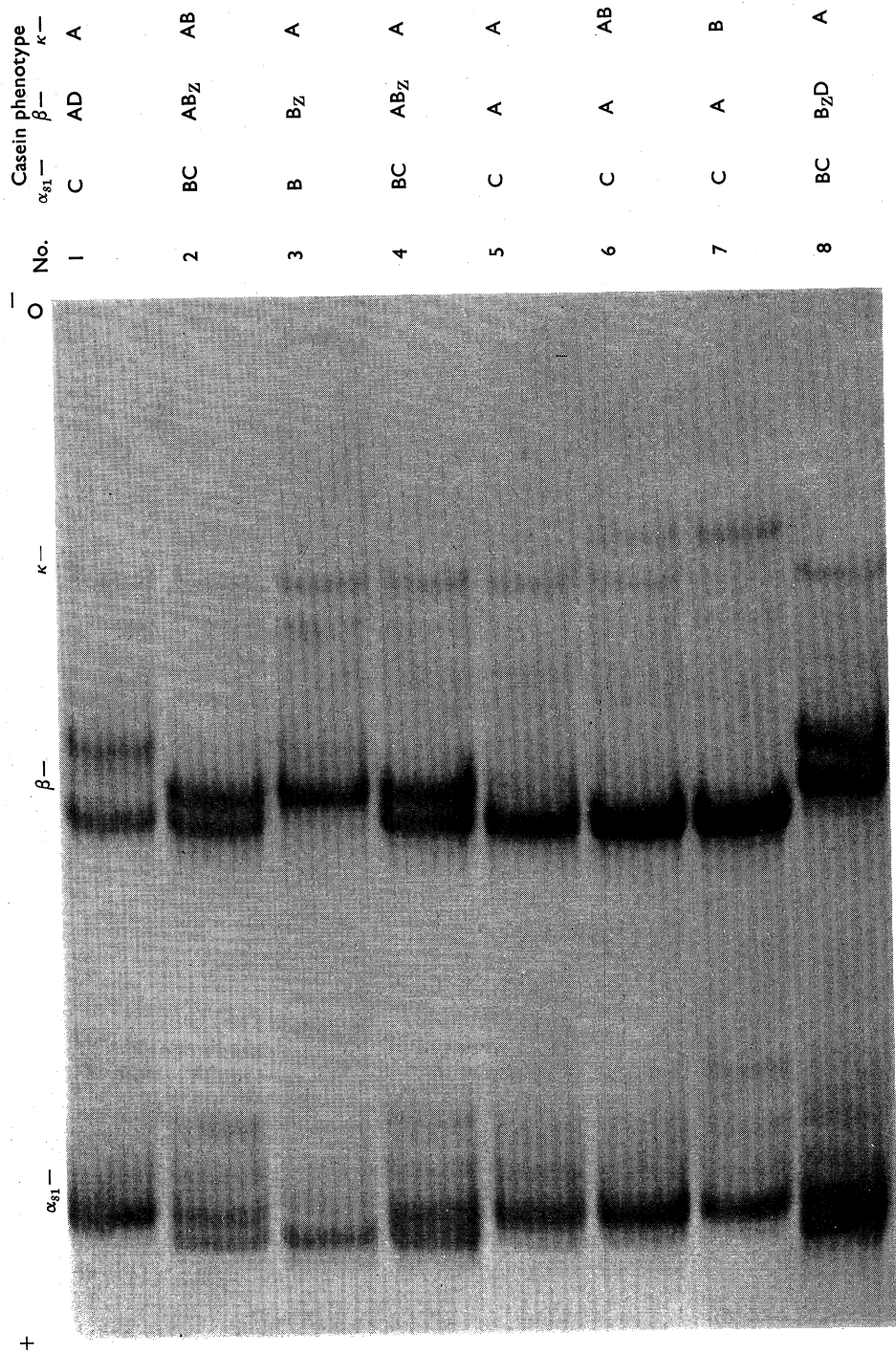


FIG. 1. Casein phenotypes of Zebu cattle. Polyacrylamide gel electrophoresis in alkaline buffer. O = Origin. β-casein D is the new variant.

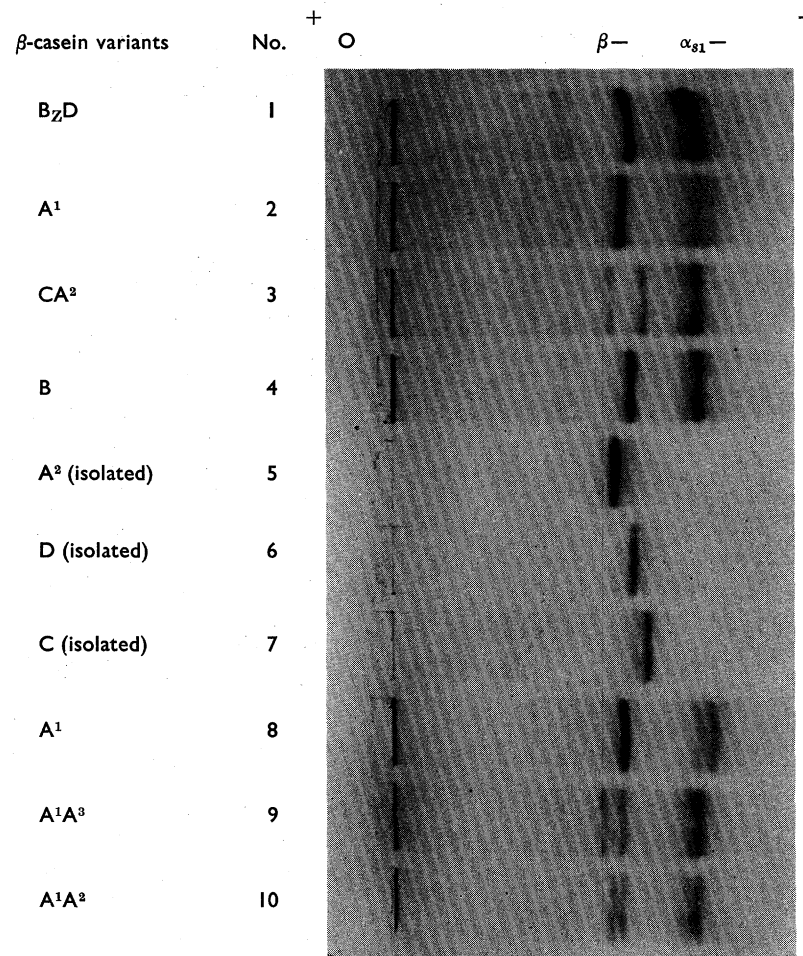


FIG. 3. Differentiation of the  $\beta$ -casein variants in acid polyacrylamide gel. O = origin. Nos. 1, 5, 6 and 10 are from Zebu cows, the others from Western cattle.

The remaining two were of phenotype  $A^1A^2$ . Clearly, polymorphism of  $\beta$ -casein A is quite rare in Indian Zebu cattle, but it was found to be far more pronounced in the East African Ankole cows (Table 2). Unfortunately the milk samples from the Boran cows were no longer extant when the procedures of acid-gel electrophoresis became available.

TABLE 2— $\beta$ -CASEIN PHENOTYPES OF FORTY-NINE\* ANKOLE CATTLE†

Phenotype	$A^1$	$A^1A^2$	$A^2$	$A^1B_Z$	$A^2B_Z$	$B_Z$
No. observed	3	21	23	2	0	0
No. expected‡	4.3	19.8	22.8	0.6	1.4	0.02

$\chi^2$  (2 d.f.) = 5.1.

\* One of the fifty milk samples was accidentally lost.

† Results obtained by TSGE in acid buffer.

‡ From Hardy-Weinberg law.

The East African samples were collected in the form of fresh milk so that the phenotypes of the whey proteins,  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin, could also be determined. The principal object of this additional work was to make a careful search for the occurrence of the C-variant of  $\beta$ -lactoglobulin which is characteristic of the Jersey breed (Bell, 1962; Aschaffenburg, 1965). It was felt that detection of this variant in Zebu cattle would strengthen the rather speculative hypothesis (Boston, 1954) of the Zebu origin of the Jersey. The search proved fruitless; no variants other than A and B were found. There was a predominance of  $\beta$ -lactoglobulin B, with gene frequencies of 0.93 for the Boran, and 0.98 for the Ankole cows. There was likewise little variation in  $\alpha$ -lactalbumin, the gene frequencies of the A-variant, found only in Zebu cattle (Blumberg & Tombs, 1958; Aschaffenburg, 1963; Bhattacharya *et al.*, 1963), being very low for the Boran (0.05) and for the Ankole cattle (0.04).

#### DISCUSSION

Excepting one instance ( $\beta$ -casein  $B_Z$ ), our results will be discussed on the presumption that the casein variants found in both Zebu and Western cattle are identical if their electrophoretic mobilities are identical. We are aware that this is no more than a reasonable assumption, but one strengthened by the findings of identity of the chymotryptic digests of  $\alpha_{s1}$ -casein C and  $\beta$ -casein  $A^2$ . Clearly, however, strict proof of the validity of this presumption will have to be obtained for each of the remaining constituents.

In view of the close kinship between *Bos indicus* and *Bos taurus*, the finding that Zebu cattle, like those of Western breeds, produce genetic variants of  $\alpha_{s1}$ -,  $\beta$ - and  $\kappa$ -casein in their milk is hardly surprising. That the earlier examination of a few Indian milk samples (Aschaffenburg & Sen, 1963) failed to produce evidence of polymorphism can be explained by the present results showing that few animals

breeds originated from the Far East. On the other hand, the almost complete reversal in the gene frequencies of the  $\alpha_{s1}$ -alleles could be regarded as evidence against this hypothesis.

The association between  $\alpha_{s1}$ -Cn<sup>C</sup> and  $\beta$ -Cn<sup>A</sup>, on the one hand, and between  $\alpha_{s1}$ -Cn<sup>B</sup> and  $\beta$ -Cn<sup>B<sub>Z</sub></sup>, on the other, is remarkably close for the Indian Zebu, and we were surprised that this does not hold for the 104 African Zebu cows:

		A	AB <sub>Z</sub>	B <sub>Z</sub>	AD
	B	15	1	—	—
$\alpha_{s1}$ -casein	BC	44	5	—	1
	C	35	1	—	2

These data resemble those for Western breeds in the occurrence of the combination  $\alpha_{s1}$ -Cn<sup>B</sup>/ $\beta$ -Cn<sup>A</sup> which is rarely found in the Indian material.

As shown above, the new variant,  $\beta$ -casein D, occurs in combination with  $\alpha_{s1}$ -casein C in both the Indian (Deshi) and African (Boran) cows. Thus, unlike  $\beta$ -casein B<sub>Z</sub>, the D-variant appears to associate readily with  $\alpha_{s1}$ -casein C.

Like the  $\beta$ -casein C of Western cattle which is found only in certain breeds, e.g. the Guernsey and Brown Swiss (King *et al.*, 1965), and in low frequency, the new variant D seems to be rare and restricted to some breeds of Zebu cows. Its very rarity enhances its value as a "marker", and it is felt that considerable significance can be attached to its discovery in milks from the African Boran as well as in those from Indian Deshi cows, a finding giving strong support to the contention that African cattle originated from the Far East.

While significance can be attached to positive evidence of this kind, the opposite is not necessarily true. The failure to detect the presence of  $\beta$ -lactoglobulin C in the milk of East African cows cannot be regarded as conclusive evidence of the absence of this variant from Zebu milk. Firstly, only two of the numerous types of Zebu cattle were available for phenotyping. Secondly, samples were relatively few in numbers. It is worth remembering that no more than two heterozygotes for  $\beta$ -lactoglobulin C were observed by one of us in an examination of eighty Jersey milks (Aschaffenburg, 1965). Clearly a wider search for this rare variant is called for.

Also, it is evident that further, more extensive, surveys should be made of the milk protein phenotypes occurring in Indian and African Zebu breeds which are far more numerous than we have been able to cover in the present work. Preferably future surveys should be combined with those of other genetic systems, e.g. serum transferrins and, in particular, blood groups which, as stressed by Stormont (1962), are of great potential value in tracing the origin of, and relationship between, breeds. Results obtained in this way would surely repay the efforts required for the organization of more complex schemes of this kind.

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